Yeasts

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Glossary

bioethanol Ethyl alcohol produced by yeast fermentation for use as a renewable biofuel.
birth scar Concave indentations that remain on the surface of daughter cells following budding.
budding A mode of vegetative reproduction in many yeast species in which a small outgrowth, the daughter bud, grows from the surface of a mother cell and eventually separates to form a new cell during cell division.
bud scar The chitin-rich, convex, ringed protrusions that remain on the mother cell surface of budding yeasts following the birth of daughter cells.
Candida albicans Common opportunistic human pathogenic yeast causing candidosis.
Crabtree effect The suppression of yeast respiration by high levels of glucose. This phenomenon is found in *Saccharomyces cerevisiae* cells, which continue to ferment irrespective of oxygen availability due to glucose repressing or inactivating the respiratory enzymes or due to the inherent limited capacity of cells to respire.
fission A mode of vegetative reproduction found in the yeast genus *Schizosaccharomyces*. Fission yeasts grow lengthwise and divide by forming a cell septum that constricts mother cells into two equal-sized daughters.
Pasteur effect Under anaerobic conditions, glycolysis proceeds faster than it does under aerobic conditions. In *Saccharomyces cerevisiae*, the Pasteur effect is observable only when the glucose concentration is low ($< \sim 5$ mM) or in nutrient-deficient cells.
respirofermentation Fermentative metabolism of yeast in the presence of oxygen.
Saccharomyces cerevisiae Baker's or brewer's yeast species, which is used widely in the food and fermentation industries and is also being exploited in modern biotechnology (e.g., in the production of recombinant proteins) and as a model eukaryotic cell in fundamental biological research.
sporulation The production of haploid spores when sexually reproductive yeasts conjugate and undergo meiosis.

Abbreviations

AFLP	amplified fragment length polymorphism
AFM	Atomic force microscopy
CDI	cyclin-dependent kinase inhibitor
DEAE	diethylaminoethyl
ER	endoplasmic reticulum
FACS	Fluorescence-activated cell sorting
GAP	general amino acid permease
NAD	nicotinamide adenine dinucleotide
RAPD	random amplified polymorphic DNA
YEPG	yeast extract peptone glucose
YNB	yeast nitrogen base

Defining Statement

Yeasts are eukaryotic unicellular microfungi that play important roles in industry, the environment, and medical science. This article describes the classification, ecology, cytology, metabolism, and genetics of yeast, with specific reference to *Saccharomyces cerevisiae* – baker's yeast. The biotechnological potential of yeasts, including their exploitation in food, fermentation, and pharmaceutical industries, is also discussed in the article.

Definition and Classification of Yeasts

Definition and Characterization of Yeasts

Yeasts are recognized as unicellular fungi that reproduce primarily by budding, and occasionally by fission, and that do not form their sexual states (spores) in or on a fruiting body. Yeast species may be identified and characterized according to various criteria based on cell morphology (e.g., mode of cell division and spore shape), physiology (e.g., sugar fermentation tests), immunology (e.g., immunofluorescence), and molecular biology (e.g., ribosomal DNA phylogeny, DNA reassociation, DNA base composition and hybridization, karyotyping, random amplified polymorphic DNA (RAPD), and amplified fragment length polymorphism (AFLP) of D1/D2 domain sequences of 26S rDNA). Molecular sequence analyses are being increasingly used by yeast taxonomists to categorize new species.

Yeast Taxonomy

The most commercially exploited yeast species, *S. cerevisiae* (baker's yeast), belongs to the fungal kingdom subdivision Ascomycotina. **Table 1** summarizes the taxonomic hierarchy of yeasts, with *S. cerevisiae* as an example.

Other yeast genera are categorized under Basidiomycotina (e.g., *Cryptococcus* spp. and *Rhodotorula* spp.) and Deuteromycotina (e.g., *Candida* spp. and *Brettanomyces* spp.). There are around 100 recognized yeast genera and the reader is directed to Kurtzman and Fell (1998) for additional information on yeast taxonomy.

Yeast Biodiversity

Around 1000 species of yeast have been described, but new species are being characterized on a regular basis and there is considerable untapped yeast biodiversity on Earth. For example, it has been estimated (in 1996) that only 0.065% of yeast genera (total 62 000) and 0.22% of yeast species (total 669 000) have been isolated and characterized. This means that there is an immense gap in our knowledge regarding biodiversity and the available 'gene pool' of wild natural isolates of yeast. Several molecular biological techniques are used to assist in the detection of new yeast species in the natural environment, and together with input from cell physiologists, they provide ways to conserve and exploit yeast biodiversity. *S. cerevisiae* is the most studied and exploited of all the yeasts, but the biotechnological potential of non-*Saccharomyces* yeasts is gradually being realized, particularly with regard to recombinant DNA technology (see **Table 8**).

Table 1 Taxonomic hierarchy of yeast

Taxonomic category	*Example (*Saccharomyces cerevisiae*)*
Kingdom	Fungi
Division	Ascomycota
Subdivision	Ascomycotina
Class	Hemiascomycete
Order	Endomycetales
Family	Saccharomycetacae
Subfamily	Saccharomyetoideae
Genus	*Saccharomyces*
Species	*cerevisiae*

Yeast Ecology

Natural Habitats of Yeast Communities

Yeasts are not as ubiquitous as bacteria in the natural environment, but nevertheless they can be isolated from soil, water, plants, animals, and insects. Preferred yeast habitats are plant tissues (leaves, flowers, and fruits), but a few species are found in commensal or parasitic relationships with animals. Some yeasts, most notably *Candida albicans*, are opportunistic human pathogens. Several species of yeast may be isolated from specialized or extreme environments, such as those with low water potential (i.e., high sugar or salt concentrations), low temperature (e.g., some psychrophilic yeasts have been isolated from polar regions), and low oxygen availability (e.g., intestinal tracts of animals). **Table 2** summarizes the main yeast habitats.

Yeasts in the Food Chain

Yeasts play important roles in the food chain. Numerous insect species, notably *Drosophila* spp., feed on yeasts that colonize plant material. As insect foods, ascomycetous yeasts convert low-molecular-weight nitrogenous compounds into proteins beneficial to insect nutrition. In addition to providing a food source, yeasts may also affect the physiology and sexual reproduction of drosophilids. In marine environments, yeasts may serve as food for filter feeders.

Microbial Ecology of Yeasts

In microbial ecology, yeasts are not involved in biogeochemical cycling as much as bacteria or filamentous fungi. Nevertheless, yeasts can use a wide range of carbon sources and thus play an important role as saprophytes in the carbon cycle, degrading plant detritus to carbon dioxide. In the cycling of nitrogen, some yeasts can reduce nitrate or ammonify nitrite, although most yeasts assimilate ammonium ions or amino acids into organic nitrogen. Most yeasts can reduce sulfate, although some are sulfur auxotrophs.

Table 2 Natural yeast habitats

Habitat	*Comments*
Soil	Soil may only be a reservoir for the long-term survival of many yeasts, rather than a habitat for growth. However, yeasts are ubiquitous in cultivated soils (about 10 000 yeast cells per gram of soil) and are found only in the upper, aerobic soil layers (10–15 cm). Some genera are isolated exclusively from soil (e.g., *Lipomyces* and *Schwanniomyces*)
Water	Yeasts predominate in surface layers of fresh and salt waters, but are not present in great numbers (about 1000 cells per liter). Many aquatic yeast isolates belong to red pigmented genera (*Rhodotorula*). *Debaryomyces hansenii* is a halotolerant yeast that can grow in nearly saturated brine solutions
Atmosphere	A few viable yeast cells may be expected per cubic meter of air. From layers above soil surfaces, *Cryptococcus*, *Rhodotorula*, *Sporobolomyces*, and *Debaryomyces* spp. are dispersed by air currents
Plants	The interface between soluble nutrients of plants (sugars) and the septic world are common niches for yeasts (e.g., the surface of grapes); the spread of yeasts on the phyllosphere is aided by insects (e.g., *Drosophila* spp.); a few yeasts are plant pathogens. The presence of many organic compounds on the surface and decomposing areas (exudates, flowers, fruits, phyllosphere, rhizosphere, and necrotic zones) creates conditions favorable for growth of many yeasts
Animals	Several nonpathogenic yeasts are associated with the intestinal tract and skin of warm-blooded animals; several yeasts (e.g., *Candida albicans*) are opportunistically pathogenic toward humans and animals; numerous yeasts are commensally associated with insects, which act as important vectors in the natural distribution of yeasts
Built environment	Yeasts are fairly ubiquitous in buildings, for example, *Aureobasidium pullulans* (black yeast) is common on damp household wallpaper and *Saccharomyces cerevisiae* is readily isolated from surfaces (pipework and vessels) in wineries

Yeast Cell Structure

General Cellular Characteristics

Yeasts are unicellular eukaryotes that have ultrastructural features similar to that of higher eukaryotic cells. This, together with their ease of growth, and amenability to biochemical, genetic, and molecular biological analyses, makes yeasts model organisms in studies of eukaryotic cell biology. Yeast cell size can vary widely, depending on the species and conditions of growth. Some yeasts may be only 2–3 μm in length, whereas others may attain lengths of 20–50 μm. Cell width appears less variable, between 1 and 10 μm. *S. cerevisiae* is generally ellipsoid in shape with a large diameter of 5–10 μm and a small diameter of 1–7 μm. **Table 3** summarizes the diversity of yeast cell shapes.

Table 3 Diversity of yeast cell shapes

Cell shape	*Description*	*Examples of yeast genera*
Ellipsoid	Ovoid-shaped cells	*Saccharomyces*
Cylindrical	Elongated cells with hemispherical ends	*Schizosaccharomyces*
Apiculate	Lemon shaped	*Hanseniaspora*, *Saccharomycodes*
Ogival	Elongated cell rounded at one end and pointed at other	*Dekkera*, *Brettanomyces*
Flask shaped	Cells dividing by bud fission	*Pityrosporum*
Pseudohyphal	Chains of budding yeast cells, which have elongated without detachment. Pseudohyphal morphology is intermediate between a chain of yeast cells and a hypha	Occasionally found in starved cells of *Saccharomyces cerevisiae* and frequently in *Candida albicans* (filamentous cells form from 'germ tubes', and hyphae may give rise to buds called blastospores)
Hyphal	Basidiomycetous yeast cells grow lengthwise to form branched or unbranched threads or true hyphae, occasionally with septa (cross walls) to make up mycelia. Septa may be laid down by the continuously extending hyphal tip	*Saccharomycopsis* spp.
Dimorphic	Yeasts that grow vegetatively in either yeast or filamentous forms	*C. albicans*, *Saccharomycopsis fibuligera*, *Kluyveromyces marxianus*, *Malassezia furfur*, *Yarrowia lipolytica*, *Ophiostoma novo-ulmi*, *Sporothrix schenkii*, *Histoplasma capsulatum*
Miscellaneous		
	Triangular	*Trigonopsis*
	Curved	*Cryptococcus*
	Stalked	*Sterigmatomyces*
	Spherical	*Debaryomyces*

Several yeast species are pigmented and various colors may be visualized in surface-grown colonies, for example, cream (e.g., *S. cerevisiae*), white (e.g., *Geotrichum* spp.), black (e.g., *Aureobasidium pullulans*), pink (e.g., *Phaffia rhodozyma*), red (e.g., *Rhodotorula* spp.), orange (e.g., *Rhodosporidium* spp.), and yellow (e.g., *Bullera* spp.). Some pigmented yeasts have applications in biotechnology. For example, the astaxanthin pigments of *P. rhodozyma* have applications as fish feed colorants for farmed salmonids, which have no means of synthesizing these red compounds.

Methods in Yeast Cytology

By using various cytochemical and cytofluorescent dyes and phase contrast microscopy, it is possible to visualize several subcellular structures in yeasts (e.g., cell walls, capsules, nuclei, vacuoles, mitochondria, and several cytoplasmic inclusion bodies). The *GFP* gene from the jellyfish (*Aequorea victoria*) encodes the green fluorescent protein (which fluoresces in blue light) and can be used to follow the subcellular destiny of certain expressed proteins when GFP is fused with the genes of interest. Immunofluorescence can also be used to visualize yeast cellular features when dyes such as fluorescein isothiocyanate and rhodamine B are conjugated with monospecific antibodies raised against yeast structural proteins. Confocal scanning laser immunofluorescence microscopy can also be used to detect the intracellular localization of proteins within yeast cells and to give three-dimensional ultrastructural information. Fluorescence-activated cell sorting (FACS) has proven very useful in studies of the yeast cell cycle and in monitoring changes in organelle (e.g., mitochondrial) biogenesis. Scanning electron microscopy is useful in revealing the cell surface topology of yeasts, as is atomic force microscopy, which has achieved high-contrast nanometer resolution for yeast cell surfaces (**Figure 1**). Transmission electron microscopy, however, is essential for visualizing the intracellular fine structure of ultrathin yeast cell sections (**Figure 2**).

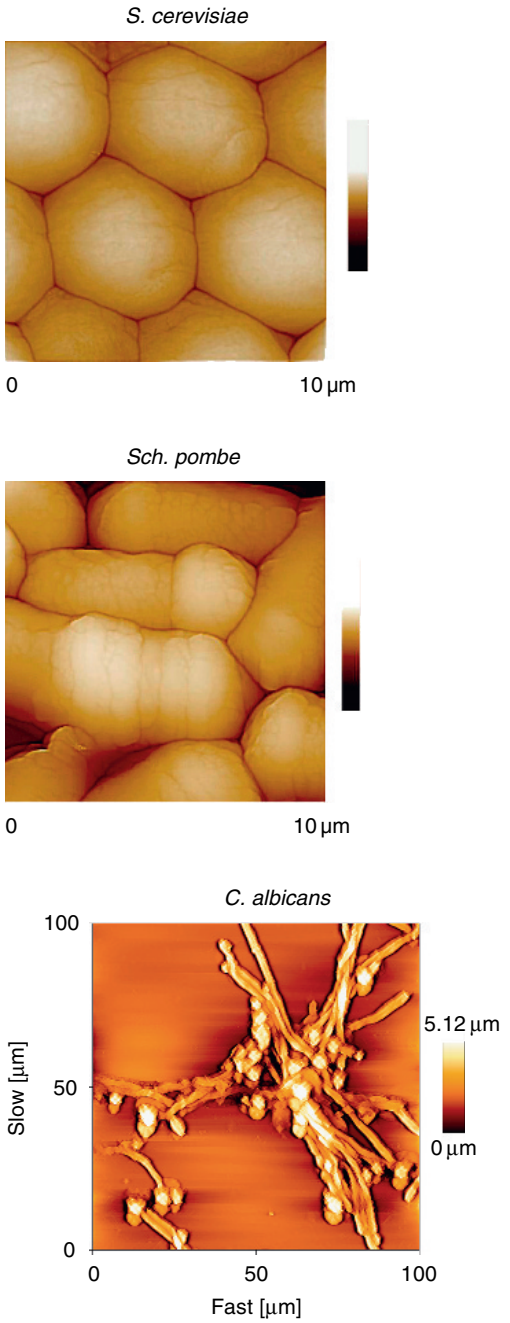

Figure 1 Atomic force microscopy (AFM) of yeast cell surfaces. Courtesy of Dr. A Adya and Dr. E Canetta, University of Abertay Dundee.

Subcellular Yeast Architecture and Function

Transmission electron microscopy of a yeast cell typically reveals the cell wall, nucleus, mitochondria, endoplasmic reticulum (ER), Golgi apparatus, vacuoles, microbodies, and secretory vesicles. **Figure 2** shows an electron micrograph of a typical yeast cell.

Several of these organelles are not completely independent of each other and derive from an extended intramembranous system. For example, the movement and positioning of organelles depends on the cytoskeleton, and the trafficking of proteins in and out of cells relies on vesicular communication between the ER, Golgi apparatus, vacuole, and plasma membrane. Yeast organelles can be readily isolated for further studies by physical, chemical, or enzymatic disruption of the cell wall, and the purity of organelle preparations can be evaluated using specific marker enzyme assays.

In the yeast cytoplasm, ribosomes and occasionally plasmids (e.g., 2 µm circles) are found, and the structural

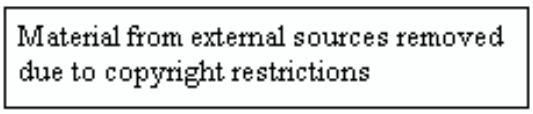

Figure 2 Ultrastructural features of a yeast cell. The transmission electron micrograph is of a *Candida albicans* cell. BS, bud scar; CM, cell membrane; CMI, cell membrane invagination; CW, cell wall; ER, endoplasmic reticulum; M, mitochondrion; N, nucleus; and V, vacuole. Courtesy of M Osumi, Japan Women's University, Tokyo.

organization of the cell is maintained by a cytoskeleton of microtubules and actin microfilaments. The yeast cell envelope, which encases the cytoplasm, comprises (from the inside looking out) the plasma membrane, periplasm, cell wall, and, in certain yeasts, a capsule and a fibrillar layer. Spores encased in an ascus may be revealed in those yeasts that undergo differentiation following sexual conjugation and meiosis. **Table 4** provides a summary of the physiological functions of the various structural components found in yeast cells.

Nutrition, Metabolism, and Growth of Yeasts

Nutritional and Physical Requirements for Yeast Growth

Yeast nutritional requirements

Yeast cells require macronutrients (sources of carbon, nitrogen, oxygen, sulfur, phosphorus, potassium, and magnesium) at the millimolar level in growth media, and they require trace elements (e.g., Ca, Cu, Fe, Mn, and Zn) at the micromolar level. Most yeasts grow quite well in simple nutritional media, which supply carbon–nitrogen backbone compounds together with inorganic ions and a few growth factors. Growth factors are organic compounds required in very low concentrations for specific catalytic or structural roles in yeast, but are not used as energy sources. Yeast growth factors include vitamins, which serve vital functions as components of coenzymes; purines and pyrimidines; nucleosides and nucleotides; amino acids; fatty acids; sterols; and other miscellaneous compounds (e.g., polyamines and choline). Growth factor requirements vary among yeasts, but when a yeast species is said to have a growth factor requirement, it indicates that the species cannot synthesize the particular factor, resulting in the curtailment of growth without its addition to the culture medium.

Yeast culture media

It is quite easy to grow yeasts in the laboratory on a variety of complex and synthetic media. Malt extract or yeast extract supplemented with peptone and glucose (as

Table 4 Functional components of an ideal yeast cell

Organelle or cellular structure	*Function*
Cell envelope	Comprises the plasma membrane that acts as a selectively permeable barrier for transport of hydrophilic molecules in and out of fungal cells; the periplasm containing proteins and enzymes unable to permeate the cell wall; the cell wall that provides protection and shape and is involved in cell–cell interactions, signal reception, and specialized enzyme activities; fimbriae involved in sexual conjugation; and capsules to protect cells from dehydration and immune cell attack
Nucleus	Contains chromosomes (DNA–protein complexes) that pass genetic information to daughter cells during cell division and the nucleolus, which is the site of ribosomal RNA transcription and processing
Mitochondria	Responsible, under aerobic conditions, for respiratory metabolism and, under anaerobic conditions, for fatty acid, sterol, and amino acid metabolism
Endoplasmic reticulum	Ribosomes on the rough endoplasmic reticulum are the sites of protein biosyntheses (translation of mRNA nucleotide sequences into amino acid sequences in a polypeptide chain)
Proteasome	Multi-subunit protease complexes involved in regulating protein turnover
Golgi apparatus and vesicles	Secretory system for import (endocytosis) and export (exocytosis) of proteins
Vacuole	Intracellular reservoir (amino acids, polyphosphate, and metal ions), proteolysis, protein trafficking, and control of intracellular pH
Peroxisome	Present in some methylotrophic (methanol-utilizing) yeasts for oxidative utilization of specific carbon and nitrogen sources (contain catalase and oxidases). Glyoxysomes contain enzymes of the glyoxylate cycle

Reproduced from Walker GM and White NA (2005) Introduction to fungal physiology. In: Kavanagh K (ed.) *Fungi: Biology and Applications*, ch. 2, pp. 1–34. Chichester, UK: John Wiley & Sons.

in YEPG) is commonly employed for the maintenance and growth of most yeasts. Yeast nitrogen base (YNB) is a commercially, available chemically defined medium that contains ammonium sulfate and asparagine as nitrogen sources, together with mineral salts, vitamins, and trace elements. The carbon source of choice (e.g., glucose) is usually added to a final concentration of 1% (w/v). For the continuous cultivation of yeasts in chemostats, media that ensure that all the nutrients for growth are present in excess except one (the growth-limiting nutrient) are usually designed. Chemostats can therefore facilitate studies on the influence of a single nutrient (e.g., glucose, in carbon-limited chemostats) on yeast cell physiology, with all other factors being kept constant. In industry, yeasts are grown in a variety of fermentation feedstocks, including malt wort, molasses, grape juice, cheese whey, glucose syrups, and sulfite liquor.

Physical requirements for yeast growth

Most yeast species thrive in warm, dilute, sugary, acidic, and aerobic environments. Most laboratory and industrial yeasts (e.g., *S. cerevisiae* strains) grow best from 20 to 30 °C. The lowest maximum temperature for growth of yeasts is around 20 °C, whereas the highest is around 50 °C.

Yeasts need water in high concentration for growth and metabolism. Several food spoilage yeasts (e.g., *Zygosaccharomyces* spp.) are able to withstand conditions of low water potential (i.e., high sugar or salt concentrations), and such yeasts are referred to as osmotolerant or xerotolerant.

Most yeasts grow very well between pH 4.5 and 6.5. Media acidified with organic acids (e.g., acetic and lactic) are more inhibitory to yeast growth than are media acidified with mineral acids (e.g., hydrochloric). This is because undissociated organic acids can lower intracellular pH following their translocation across the yeast cell membrane. This forms the basis of the action of weak acid preservatives in inhibiting food spoilage yeast growth. Actively growing yeasts acidify their growth environment through a combination of differential ion uptake, proton secretion during nutrient transport (see later), direct secretion of organic acids (e.g., succinate and acetate), and carbon dioxide evolution and dissolution. Intracellular pH is regulated within relatively narrow ranges in growing yeast cells (e.g., around pH 5 in *S. cerevisiae*), mainly through the action of the plasma membrane proton-pumping ATPase.

Most yeasts are aerobes. Yeasts are generally unable to grow well under completely anaerobic conditions because, in addition to providing the terminal electron acceptor in respiration, oxygen is needed as a growth factor for membrane fatty acid (e.g., oleic acid) and sterol (e.g., ergosterol) biosynthesis. In fact, *S. cerevisiae* is auxotrophic for oleic acid and ergosterol under anaerobic conditions and this yeast is not, strictly speaking, a facultative anaerobe. **Table 5** categorizes yeasts based on their fermentative properties and growth responses to oxygen availability.

Carbon Metabolism by Yeasts

Carbon sources for yeast growth

As chemorganotrophic organisms, yeasts obtain carbon and energy in the form of organic compounds. Sugars are widely used by yeasts. *S. cerevisiae* can grow well on glucose, fructose, mannose, galactose, sucrose, and maltose. These sugars are also readily fermented into ethanol and carbon dioxide by *S. cerevisiae*, but other carbon substrates such as ethanol, glycerol, and acetate can be respired by *S. cerevisiae* only in the presence of oxygen. Some yeasts (e.g., *Pichia stipitis* and *Candida shehatae*) can use five-carbon pentose sugars such as D-xylose and L-arabinose as growth and fermentation substrates. A few amylolytic yeasts (e.g., *Saccharomyces diastaticus* and *Schwanniomyces occidentalis*) that can use starch exist, and several oleaginous yeasts (e.g., *Candida tropicalis* and *Yarrowia lipolytica*) can grow on hydrocarbons, such as straight-chain alkanes in the C_{10}–C_{20} range. Several methylotrophic yeasts (e.g., *Hansenula polymorpha* and *Pichia pastoris*) can grow very well on methanol as the sole carbon and energy source, and these yeasts have

Table 5 Classification of yeasts based on fermentative property/growth response to oxygen availability

Class	*Examples*	*Comments*
Obligately fermentative	*Candida pintolopesii (Saccharomyces telluris)*	Naturally occurring respiratory-deficient yeasts. Only ferment, even in the presence of oxygen
Facultatively fermentative		
Crabtree-positive	*Saccharomyces cerevisiae*	Such yeasts predominantly ferment high-sugar-containing media in the presence of oxygen (respirofermentation)
Crabtree-negative	*Candida utilis*	Such yeasts do not form ethanol under aerobic conditions and cannot grow anaerobically
Nonfermentative	*Rhodotorula rubra*	Such yeasts do not produce ethanol, in either the presence or absence of oxygen

industrial potential in the production of recombinant proteins using methanol-utilizing genes as promoters.

Yeast sugar transport

Sugars are transported into yeast cells across the plasma membrane by various mechanisms such as simple net diffusion (a passive or free mechanism), facilitated (catalyzed) diffusion, and active (energy-dependent) transport. The precise mode of sugar translocation will depend on the sugar, yeast species, and growth conditions. For example, *S. cerevisiae* takes up glucose by facilitated diffusion and maltose by active transport. Active transport means that the plasma membrane ATPases act as directional proton pumps in accordance with chemiosmotic principles. The pH gradients thus drive nutrient transport either via proton symporters (as is the case with certain sugars and amino acids) or via proton antiporters (as is the case with potassium ions).

Yeast sugar metabolism

The principal metabolic fates of sugars in yeasts are the dissimilatory pathways of fermentation and respiration (shown in **Figure 3**) and the assimilatory pathways of gluconeogenesis and carbohydrate biosynthesis. Yeasts described as fermentative are able to use organic substrates (sugars) anaerobically as electron donors, electron acceptors, and carbon sources. During alcoholic fermentation of sugars, *S. cerevisiae* and other fermentative yeasts reoxidize the reduced coenzyme NADH to NAD (nicotinamide adenine dinucleotide) in terminal step reactions from pyruvate. In the first of these terminal reactions, catalyzed by pyruvate decarboxylase, pyruvate is decarboxylated to acetaldehyde, which is finally reduced by alcohol dehydrogenase to ethanol. The regeneration of NAD is necessary to maintain the redox balance and prevent the stalling of glycolysis. In alcoholic beverage fermentations (e.g., of beer, wine, and distilled spirits), other fermentation metabolites, in addition to ethanol and carbon dioxide, that are very important in the development of flavor are produced by yeast. These metabolites include fusel alcohols (e.g., isoamyl alcohol), polyols (e.g., glycerol), esters (e.g., ethyl acetate), organic acids (e.g., succinate), vicinyl diketones (e.g., diacetyl), and aldehydes (e.g., acetaldehyde). The production of glycerol (an important industrial commodity) can be enhanced in yeast fermentations by the addition of sulfite, which chemically traps acetaldehyde.

$$\text{Glucose} + HSO_3^- \rightarrow \text{glycerol} + \text{acetaldehyde}{-}HSO_3^- + CO_2$$

Aerobic respiration of glucose by yeasts is a major energy-yielding metabolic route and involves glycolysis, the citric acid cycle, the electron transport chain, and oxidative phosphorylation. The citric acid cycle (or Krebs cycle) represents the common pathway for the oxidation of sugars and other carbon sources in yeasts and filamentous fungi and results in the complete oxidation of one pyruvate molecule to $2CO_2$, 3NADH, $1FADH_2$, $4H^+$, and 1GTP.

Of the environmental factors that regulate respiration and fermentation in yeast cells, the availability of glucose and oxygen is best understood and is linked to the expression of regulatory phenomena, referred to as the Pasteur effect and the Crabtree effect. A summary of these phenomena is provided in **Table 6**.

Figure 3 Overview of sugar catabolic pathways in yeast cells. Reproduced from Walker (1998) *Yeast Physiology and Biotechnology*. Chichester, UK: John Wiley & Sons Limited.

Nitrogen Metabolism by Yeasts

Nitrogen sources for yeast growth

Although yeasts cannot fix molecular nitrogen, simple inorganic nitrogen sources such as ammonium salts are widely used. Ammonium sulfate is a commonly used nutrient in yeast growth media because it provides a source of both assimilable nitrogen and sulfur. Some yeasts can also grow on nitrate as a source of nitrogen, and, if able to do so, may also use subtoxic concentrations of nitrite. A variety of organic nitrogen compounds (amino acids, peptides, purines, pyrimidines, and amines) can also provide the nitrogenous requirements of the yeast cell. Glutamine and aspartic acids are readily deaminated by yeasts and therefore act as good nitrogen sources.

Yeast transport of nitrogenous compounds

Ammonium ions are transported in *S. cerevisiae* by both high-affinity and low-affinity carrier-mediated transport systems. Two classes of amino acid uptake systems operate in yeast cells. One is broadly specific, the general amino acid permease (GAP), and effects the uptake of all naturally occurring amino acids. The other system includes a variety of transporters that display specificity for one or a small number of related amino acids. Both the general and the specific transport systems are energy dependent.

Table 6 Summary of regulatory phenomena in yeast sugar metabolism

Phenomenon	*Description*	*Examples of yeasts*
Pasteur effect	Activation of sugar metabolism by anaerobiosis	*Saccharomyces cerevisiae* (resting or starved cells)
Crabtree effect (short-term)	Rapid ethanol production in aerobic conditions due to sudden excess of glucose (that acts to inactivate respiratory enzymes)	*S. cerevisiae* and *Schizosaccharomyces pombe*
Crabtree effect (long-term)	Ethanol production in aerobic conditions when excess glucose acts to repress respiratory genes	*S. cerevisiae* and *Sch. pombe*
Custers effect	Stimulation of ethanol fermentation by oxygen	*Dekkera* and *Brettanomyces* spp.
Kluyver effect	Anaerobic fermentation of glucose, but not of certain other sugars (disaccharides)	*Candida utilis*

Yeast metabolism of nitrogenous compounds

Yeasts can incorporate either ammonium ions or amino acids into cellular protein, or these nitrogen sources can be intracellularly catabolized to serve as nitrogen sources. Yeasts also store relatively large pools of endogenous amino acids in the vacuole, most notably arginine. Ammonium ions can be directly assimilated into glutamate and glutamine, which serve as precursors for the biosynthesis of other amino acids. The precise mode of ammonium assimilation adopted by yeasts will depend mainly on the concentration of available ammonium ions and the intracellular amino acid pools. Amino acids may be dissimilated (by decarboxylation, transamination, or fermentation) to yield ammonium and glutamate, or they may be directly assimilated into proteins.

Yeast Growth

The growth of yeasts is concerned with how cells transport and assimilate nutrients and then integrate numerous component functions in the cell in order to increase in mass and eventually divide. Yeasts have proven invaluable in unraveling the major control elements of the eukaryotic cell cycle, and research with the budding yeast, *S. cerevisiae*, and the fission yeast, *Schizosaccharomyces pombe*, has significantly advanced our understanding of cell cycle regulation, which is particularly important in the field of human cancer. For example, two scientists, Leland Hartwell and Paul Nurse, were awarded the Nobel Prize for Medicine in 2002 for their pioneering studies on the control of cell division in budding and fission yeasts, respectively.

Vegetative reproduction in yeasts

Budding is the most common mode of vegetative reproduction in yeasts and is typical in ascomycetous yeasts such as *S. cerevisiae*. **Figure 4** shows a scanning electron micrograph of budding cells of *S. cerevisiae*. Yeast buds are initiated when mother cells attain a critical cell size at a time that coincides with the onset of DNA synthesis. This is followed by localized weakening of the cell wall and this, together with tension exerted by turgor pressure, allows the extrusion of the cytoplasm in an area bounded by the new cell wall material. The mother and daughter bud cell walls are contiguous during bud development. Multilateral budding is common in which daughter buds emanate from different locations on the mother cell surface. **Figure 5** shows multilateral budding in *S. cerevisiae*. In *S. cerevisiae*, cell size at division is asymmetrical, with buds being smaller than mother cells when they separate (**Figure 6**). Some yeast genera (e.g., *Hanseniaspora* and *Saccharomycodes*) undergo bipolar budding, where buds are restricted to the tips of lemon-shaped cells. Scar tissue on the yeast cell wall, known as the bud and birth scars, remain on the daughter bud and mother cells, respectively. These scars are rich in the polymer chitin and can be stained with fluorescent dyes (e.g., calcoflour white) to provide useful information regarding cellular age in *S. cerevisiae*, since the number of scars represents the number of completed cell division cycles.

Fission is a mode of vegetative reproduction typified by species of *Schizosaccharomyces*, which divide exclusively by forming a cell septum that constricts the cell into two equal-size daughters. In *Sch. pombe*, which has been used extensively in eukaryotic cell cycle studies, newly divided daughter cells grow lengthways in a monopolar fashion for about one-third of their new cell cycle. Cells then switch to bipolar growth for about three-quarters of the cell cycle until mitosis is initiated at a constant cell length stage.

Filamentous growth occurs in numerous yeast species and may be regarded as a mode of vegetative growth alternative to budding or fission. Some yeasts exhibit a propensity to grow with true hyphae initiated from germ tubes (e.g., *C. albicans*, **Figure 7**), but others (including *S. cerevisiae*) may grow in a pseudohyphal fashion when induced to do so by unfavorable conditions. Hyphal and pseudohyphal growth represent different developmental pathways in yeasts, but cells can revert to unicellular growth upon return to more conducive growth conditions. Filamentation may therefore represent an adaptation to foraging by yeasts when nutrients are scarce.

(a)

(b)

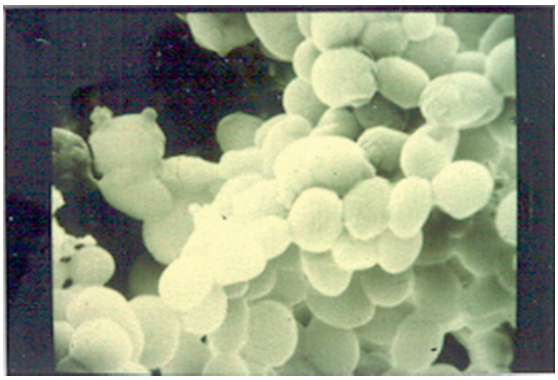

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Figure 4 Scanning electron micrographs of budding yeast. (a) Individual cell. BS, bud scar; and BirS, birth scar. Courtesy of M Osumi, Japan Women's University: Tokyo. (b) Cluster of cells.

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Figure 5 Bud scars in a single cell of *Saccharomyces cerevisiae*. The micrograph shows multilateral budding on the surface of an aged cell of *S. cerevisiae*. Courtesy of Prof. A Martini, University of Perugia, Italy.

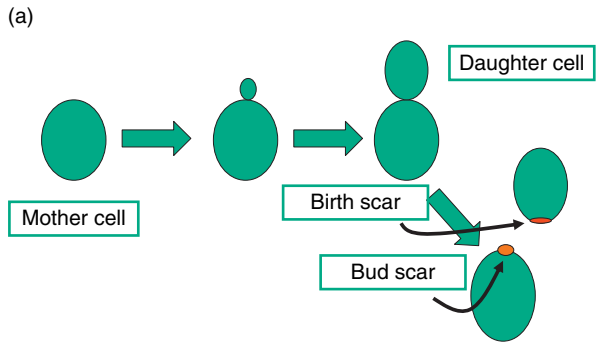
Population growth of yeasts

As in most microorganisms, when yeast cells are inoculated into a liquid nutrient medium and incubated under optimal physical growth conditions, a typical batch growth curve will result when the viable cell population is plotted against time. This growth curve is made up of a lag phase (period of no growth, but physiological adaptation of cells to their new environment), an exponential phase (limited period of logarithmic cell doublings), and a stationary phase (resting period with zero growth rate).

Diauxic growth is characterized by two exponential phases and occurs when yeasts are exposed to two carbon growth substrates that are used sequentially. This occurs during aerobic growth of *S. cerevisiae* on glucose (the second substrate being ethanol formed from glucose fermentation).

In addition to batch cultivation of yeasts, cells can also be propagated in continuous culture in which exponential growth is prolonged without lag or stationary phases. Chemostats are continuous cultures that are based on the controlled feeding of a sole growth-limiting nutrient into an open culture vessel, which permits the outflow of cells and spent medium. The feeding rate is referred to as the dilution rate, which is employed to govern the yeast growth rate under the steady-state conditions that prevail in a chemostat.

Specialized yeast culture systems include immobilized bioreactors. Yeast cells can be readily immobilized or entrapped in a variety of natural and synthetic materials (e.g., calcium alginate gel, wood chips, hydroxyapatite ceramics, diethylaminoethyl (DEAE) cellulose, or microporous



(b)

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Figure 6 Budding processes in yeast. (a) Schematic diagram of budding. (b) Budding cell cycle, as typified by *Saccharomyces cerevisiae*. S, DNA synthesis period; G_1, pre-DNA synthesis gap period; G_2, post-DNA synthesis gap period; and M, mitosis. Reproduced from Madhani H (2007) *From a to* α. *Yeast as a Model for Cellular Differentiation*. New York: Cold Spring Harbor Laboratory Press.

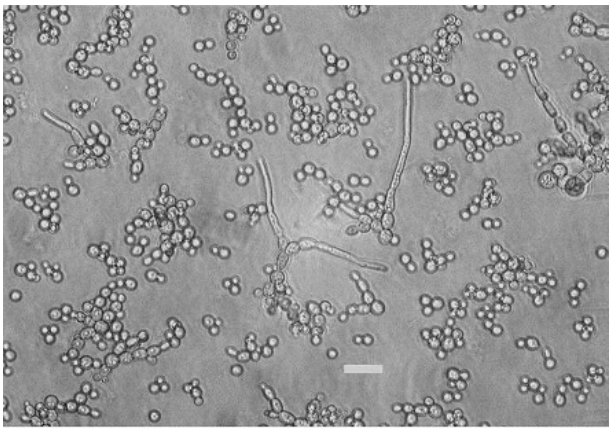

Figure 7 Dimorphism in *Candida albicans*. The micrograph shows a mixture of budding cells and hyphal forms of the yeast, which is an important human pathogen.

glass beads), and such materials have applications in the food and fermentation industries.

Yeast Genetics

Life Cycle of Yeasts

Many yeasts have the ability to reproduce sexually, but the processes involved are best understood in the budding yeast, *S. cerevisiae*, and the fission yeast, *Sch. pombe*. Both species have the ability to mate, undergo meiosis, and sporulate. The development of spores by yeasts represents a process of morphological, physiological, and biochemical differentiation of sexually reproductive cells.

Mating in *S. cerevisiae* involves the conjugation of two haploid cells of opposite mating types, designated as **a** and α (**Figure 8**). These cells synchronize one another's cell cycles in response to peptide mating pheromones, known as **a** factor and α factor.

The conjugation of mating cells occurs by cell wall surface contact followed by plasma membrane fusion to form a common cytoplasm. Karyogamy (nuclear fusion) then follows, resulting in a diploid nucleus. The stable diploid zygote continues the mitotic cell cycles in rich growth media, but if starved of nitrogen, the diploid cells sporulate to yield four haploid spores. These germinate in rich media to form haploid budding cells that can mate with each other to restore the diploid state. **Figure 9** shows mating and sporulation in *S. cerevisiae*.

In *Sch. pombe*, haploid cells of the opposite mating types (designated h^+ and h^-) secrete mating pheromones and, when starved of nitrogen, undergo conjugation to form diploids. In *Sch. pombe*, however, such diploidization is transient under starvation conditions and cells soon enter meiosis and sporulate to produce four haploid spores.

Genetic Manipulation of Yeasts

There are several ways of genetically manipulating yeast cells, including hybridization, mutation, rare mating, cytoduction, spheroplast fusion, single chromosome transfer, and transformation using recombinant DNA technology. Classic genetic approaches in *S. cerevisiae* involve mating of haploids of opposite mating types. Subsequent meiosis and sporulation result in the production of a *tetrad ascus* with four spores, which can be isolated, propagated, and genetically analyzed (i.e., tetrad analysis). This process forms the basis of genetic breeding programs for laboratory reference strains of *S. cerevisiae*. However, industrial (e.g., brewing) strains of this yeast are polyploid, are reticent to mate, and exhibit poor sporulation with low spore viability. It is, therefore, generally fruitless to perform tetrad analysis

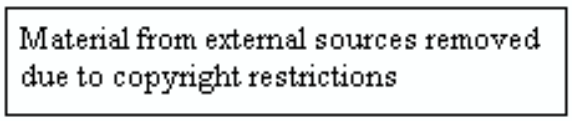

Figure 8 Sexual life cycle of *Saccharomyces cerevisiae*. Reproduced from Madhani H (2007). *From a to* α. *Yeast as a Model for Cellular Differentiation*. New York: Cold Spring Harbor Laboratory Press.

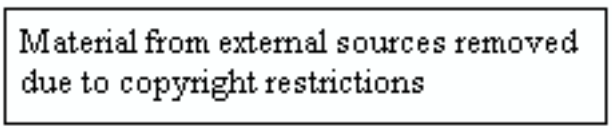

Figure 9 Meiosis and sporulation in *Saccharomyces cerevisiae*. Diploid (**a**/α) cells can undergo meiosis and sporulation to form spores that can germinate into **a** and α haploid cells. Reproduced from Madhani H (2007) *From a to* α. *Yeast as a Model for Cellular Differentiation*. New York: Cold Spring Harbor Laboratory Press.

and breeding with brewer's yeasts. Genetic manipulation strategies for preventing the sexual reproductive deficiencies associated with brewer's yeast include spheroplast fusion and recombinant DNA technology.

Intergeneric and intrageneric yeast hybrids may be obtained using the technique of spheroplast fusion. This involves the removal of yeast cell walls using lytic enzymes (e.g., glucanases from snail gut juice or microbial sources), followed by the fusion of the resulting spheroplasts in the presence of polyethylene glycol and calcium ions.

Recombinant DNA technology (genetic engineering) of yeast is summarized in **Figure 10** and transformation strategies in **Figure 11**. Yeast cells possess particular attributes for expressing foreign genes and have now become the preferred hosts, over bacteria, for producing certain human proteins for pharmaceutical use (e.g., insulin, human serum albumin, and hepatitis vaccine). Although the majority of research and development in recombinant protein synthesis in yeasts has been conducted using *S. cerevisiae*, several non-*Saccharomyces* species are being studied and exploited in biotechnology. For example, *H. polymorpha* and *P. pastoris* (both methylotrophic yeasts) exhibit particular advantages over *S. cerevisiae* in cloning technology (see **Table 8**).

Yeast Genome and Proteome Projects

A landmark in biotechnology was reached in 1996 with completion of the sequencing of the entire genome of *S. cerevisiae*. The *Sch. pombe* genome was sequenced in 2002. The functional analysis of the many orphan genes of *S. cerevisiae*, for which no function has yet been assigned, is under way through international research collaborations. Elucidation by cell physiologists of the biological function of all *S. cerevisiae* genes, that is, the complete analysis of the yeast proteome, will not only lead to an understanding of how a simple eukaryotic cell works, but also provide an insight into molecular biological aspects of heritable human disorders.

Industrial, Agricultural, and Medical Importance of Yeasts

Industrial Significance of Yeasts

Yeasts have been exploited for thousands of years in traditional fermentation processes to produce beer, wine, and bread. The products of modern yeast biotechnologies impinge on many commercially important sectors, including food, beverages, chemicals, industrial enzymes, pharmaceuticals, agriculture, and the environment (**Table 7**). *S. cerevisiae* represents the primary yeast 'cell factory' in biotechnology and is the most exploited

Figure 10 Basic procedures in yeast genetic engineering. Reproduced from Walker GM (1998). *Yeast Physiology and Biotechnology*. Chichester, UK: John Wiley & Sons.

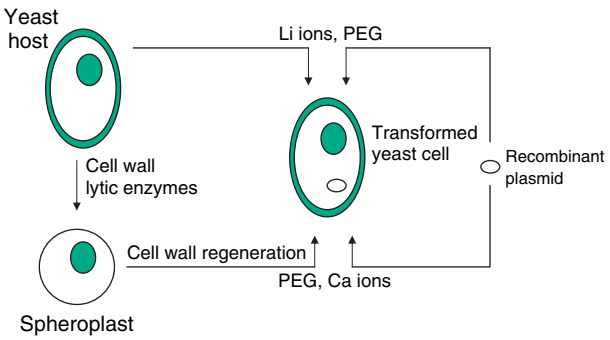

Figure 11 Yeast transformation strategies. PEG, polyethylene glycol.

Table 7 Industrial commodities produced by yeasts

Commodity	*Examples*
Beverages	Potable alcoholic beverages: Beer, wine, cider, sake, and distilled spirits (whisky, rum, gin, vodka, and cognac)
Food and animal feed	Baker's yeast, yeast extracts, fodder yeast, livestock growth factor, and feed pigments
Chemicals	Fuel ethanol (bioethanol) carbon dioxide, glycerol, and citric acid vitamins; yeasts are also used as bioreductive catalysts in organic chemistry
Enzymes	Invertase, inulinase, pectinase, lactase, and lipase
Recombinant proteins	Hormones (e.g., insulin), viral vaccines (e.g., hepatitis B vaccine), antibodies (e.g., IgE receptor), growth factors (e.g., tumor necrosis factor), interferons (e.g., leukocyte interferon-α), blood proteins (e.g., human serum albumin), and enzymes (e.g., gastric lipase and chymosin)

microorganism known, being responsible for producing potable and industrial ethanol, which is the world's premier biotechnological commodity. However, other non-*Saccharomyces* species are increasingly being used in the production of industrial commodities (**Table 8**).

Some yeasts play detrimental roles in industry, particularly as spoilage yeasts in food and beverage production (**Table 9**). Food spoilage yeasts do not cause human infections or intoxications, but do deleteriously affect food nutritive quality and are of economic importance for food producers.

In addition to their traditional roles in food and fermentation industries, yeasts are finding increasingly important roles in the environment and in the health care sector of biotechnology. Yeasts are also invaluable as model eukaryotic cells in fundamental biological and biomedical research (**Figure 12**).

Yeasts of Environmental and Agricultural Significance

A few yeast species are known to be plant pathogens. For example, *Ophiostoma novo-ulmi* is the causative agent of Dutch Elm disease, and members of the genus *Eremothecium* cause diseases such as cotton ball in plants. On the contrary, several yeasts have been shown to be beneficial to plants in preventing fungal disease. For example, *S. cerevisiae* has potential as a phytoallexin elicitor in stimulating cereal plant defenses against fungal pathogens, and several yeasts (e.g., *Cryptococcus laurentii*, *Metschnikowia pulcherrima*, *Pichia anomala*, and *Pichia guilliermondii*) may be used in the biocontrol of fungal fruit and grain spoilage, especially in preventing postharvest

Table 8 Uses of non-*Saccharomyces* yeasts in biotechnology

Yeast	*Uses*
Candida spp.	Many uses in foods, chemicals, pharmaceuticals, and xylose fermentation (*C. shehatae*)
Kluyveromyces spp.	Lactose, inulin-fermented, rich sources of enzymes (lactase, lipase, pectinase, and recombinant chymosin)
Hansenula and *Pichia*	Cloning technology. Methylotophic yeasts (*H. polymorpha* and *P. pastoris*)
Saccharomycopsis and *Schwanniomyces*	Amylolytic yeasts (starch-degrading)
Schizosaccharomyces	Cloning technology, fuel alcohol, some beverages (rum), and biomass protein
Starmerella	Wine flavor during fermentation
Yarrowia	Protein from hydrocarbons (*Y. lipolytica*)
Zygosaccharomyces	High salt/sugar fermentations (soy sauce)

Table 9 Some yeasts important in food production and food spoilage

Yeast genus	*Importance in foods*
Candida spp.	Some species (e.g., *C. utilis*, *C. guilliermondii*) are used in the production of microbial biomass protein, vitamins, and citric acid. Some species (e.g., *C. zeylanoides*) are food spoilers in frozen poultry
Cryptococcus spp.	Some strains are used as biocontrol agents to combat fungal spoilage of postharvest fruits. *C. laurentii* is a food spoilage yeast (poultry)
Debaryomyces spp.	*D. hansenii* is a salt-tolerant food spoiler (e.g., meats and fish). Also used in biocontrol of fungal fruit diseases
Kluyveromyces spp.	Lactose-fermenting yeasts are used to produce potable alcohol from cheese whey (*K. marxianus*). Source of food enzymes (pectinase, microbial rennet, and lipase) and found in cocoa fermentations. Spoilage yeast in dairy products (fermented milks and yoghurt)
Metschnikowia spp.	*M. pulcherrimia* is used in biocontrol of fungal fruit diseases (post-harvest). Osmotolerant yeasts
Phaffia spp.	*P. rhodozyma* is a source of astaxanthin food colorant used in aquaculture (feed for salmonids)
Pichia spp.	Production of microbial biomass protein, riboflavin (*P. pastoris*). *P. membranefaciens* is an important surface film spoiler of wine and beer
Rhodotorula spp.	*R. glutinis* is used as a source of food enzymes such as lipases. Some species are food spoilers of dairy products
Saccahromyces spp.	*S. cerevisiae* is used in traditional food and beverage fermentations (baking, brewing, winemaking, etc.), source of savory food extracts, and food enzymes (e.g., invertase). Also used as fodder yeast (livestock growth factor). *S. Bayanus* is used in sparkling wine fermentations, *S. diastaticus* is a wild yeast spoiler of beer, and *S. boulardii* is used as a probiotic yeast
Schizosaccharomyces spp.	*Sch. pombe* is found in traditional African beverages (sorghum beer), rum fermentations from molasses, and may be used for wine deacidification. Regarded as an osmotolerant yeast
Schwanniomyces spp.	Starch-utilizing yeasts. *Schw. castellii* may be used for production of microbial biomass protein from starch
Yarrowia spp.	*Y. lipolytica* is used in production of microbial biomass protein, citric acid, and lipases
Zygosaccharomyces spp.	*Z. rouxii* and *Z. bailii*, being osmotolerant, are important food and beverage (e.g., wine) spoilage yeasts. *Z. rouxii* is also used in soy sauce production

fungal deterioration. Other environmental benefits of yeasts are to be found in aspects of pollution control. For example, yeasts can effectively biosorb heavy metals and detoxify chemical pollutants from industrial effluents. Some yeasts (e.g., *Candida utilis*) can effectively remove carbon and nitrogen from organic wastewater.

In agriculture, live cultures of *S. cerevisiae* have been shown to stabilize the rumen environment of ruminant animals (e.g., cattle) and improve the nutrient availability to increase animal growth or milk yields. The yeasts may be acting to scavenge oxygen and prevent oxidative stress to rumen bacteria, or they may provide malic and other dicarboxylic acids to stimulate rumen bacterial growth.

Medical Significance of Yeasts

The vast majority of yeasts are beneficial to human life. However, some yeasts are opportunistically pathogenic toward humans. Mycoses caused by *C. albicans*, collectively referred to as candidosis (candidiasis), are the most common opportunistic yeast infections. There are many predisposing factors to yeast infections, but immunocompromised individuals appear particularly susceptible to candidosis. *C. albicans* infections in AIDS patients are frequently life-threatening.

The beneficial medical aspects of yeasts are apparent in the provision of novel human therapeutic agents through yeast recombinant DNA technology (see **Table 7**). Yeasts are also extremely valuable as

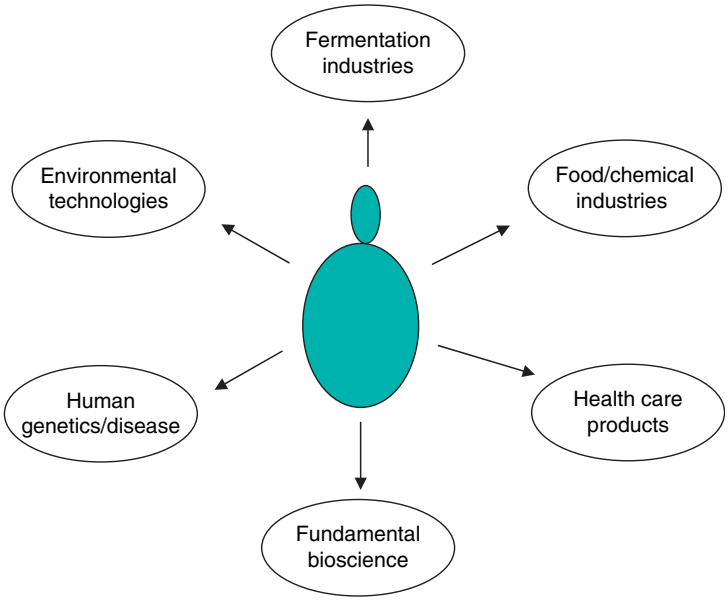

Figure 12 Uses of yeasts in biotechnology.

Table 10 Value of yeasts in biomedical research

Biomedical field	*Examples*
Oncology	Basis of cell cycle control, human oncogene(e.g., Ras) regulation; telomere function, tumor suppressor function, and design of (cyclin-dependent kinase inhibitors) CDIs/anti-cancer drugs
Aging	Mechanisms of cell aging, longevity genes, and apoptosis
Pharmacology	Multidrug resistance, drug action/metabolism, and drug screening assays
Virology	Viral gene expression, antiviral vaccines, and prion structure/function
Human genetics	Basis of human hereditary disorders and genome/proteome projects

experimental models in biomedical research, particularly in the fields of oncology, pharmacology, toxicology, virology, and human genetics (**Table 10**).

Further Reading

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